6

PATENT COOPERATION TREATY

PCT

REC'D 19 JUL 1999
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P03949PC/ALi/mck	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE98/01352	International filing date *(day/month/year)* 08.07.1998	Priority date *(day/month/year)* 09.07.1997
International Patent Classification (IPC) or national classification and IPC_6 C 12 Q 1/00, C 12 M 1/40		
Applicant Carlsson, Thomas		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.02.1999	07.07.1999
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Carolina Gómez Lagerlöf/Els Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/01352

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☒ the international application as originally filed.

☐ the description, pages _____ , as originally filed,
pages _____ , filed with the demand,
pages _____ , filed with the letter of _____ ,
pages _____ , filed with the letter of _____ .

☐ the claims, Nos. _____ , as originally filed,
Nos. _____ , as amended under Article 19,
Nos. _____ , filed with the demand,
Nos. _____ , filed with the letter of _____ ,
Nos. _____ , filed with the letter of _____ .

☐ the drawings, ~~sheets~~/fig _____ , as originally filed,
~~sheets~~/fig _____ , filed with the demand
~~sheets~~/fig _____ , filed with the letter of _____ ,
~~sheets~~/fig _____ , filed with the letter of _____ .

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, ~~sheets~~/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/01352

V. Resoned statement under Article 35(2) with regard t novelty, inventive step or industrial applicability; citati ns and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-18	YES
	Claims		NO
Inventive step (IS)	Claims	1-18	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-18	YES
	Claims		NO

2. Citations and explanations

The claims disclose a method of regenerating a biosensor in a continuous system where the flow rate of the background fluid is increased.

Document US 4153513 discloses a method for continuous determination of a concentration of an enzyme substrate in an aqueous liquid. The sample is introduced into a flowing buffer solution, but the flow rate of the buffer solution is not increased to regenerate the biosensor as it does in the application. The document shows the general state of the art.

Thus, claims 1-18 are considered to fulfil the requirements of novelty, inventive step and industrial applicability.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
LINDGREN, Anders
Dr. Ludwig Brann Patentbyrå AB
P.O. Box 17192
S-104 62 Stockholm
SUÈDE

ANKOM
1999 -02- 01

1999 -02- 03

Date of mailing (day/month/year)	
21 January 1999 (21.01.99)	
Applicant's or agent's file reference	**IMPORTANT NOTICE**
P03949PC00	

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/SE98/01352	08 July 1998 (08.07.98)	09 July 1997 (09.07.97)

Applicant
CARLSSON, Thomas

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CA,CN,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   None

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   21 January 1999 (21.01.99) under No. WO 99/02723

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01352

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/00, C12M 1/40
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12Q, C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4153513 A (HERMANN EDELMANN ET AL), 8 May 1979 (08.05.79) -- --------	1-18

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	31 -10- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/SE 98/01352

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4153513 A	08/05/79	AT 352293 B	10/09/79
		AT 430077 A	15/02/79
		BE 858791 A	16/03/78
		CA 1086616 A	30/09/80
		CH 634108 A	14/01/83
		DD 132150 A	30/08/78
		DE 2642232 A,B,C	30/03/78
		DK 376677 A	21/03/78
		FR 2393306 A,B	29/12/78
		GB 1530847 A	01/11/78
		JP 53039198 A	10/04/78
		NL 7707199 A	22/03/78
		SE 7709357 A	21/03/78

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/00, C12M 1/40 — A1

(11) International Publication Number: **WO 99/02723**

(43) International Publication Date: 21 January 1999 (21.01.99)

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(22) International Filing Date: 8 July 1998 (08.07.98)

(30) Priority Data:
9702658-7 9 July 1997 (09.07.97) SE

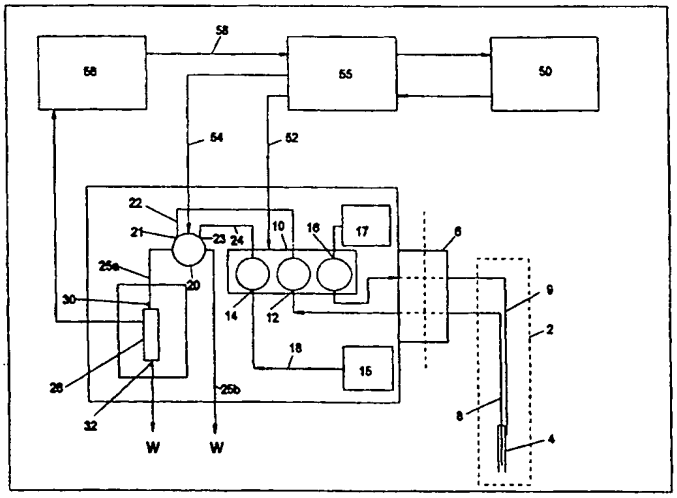
(71)(72) Applicant and Inventor: CARLSSON, Thomas [SE/SE]; Flöjtvägen 59, S-756 54 Uppsala (SE).

(74) Agents: LINDGREN, Anders et al.; Dr. Ludwig Brann Patentbyrå AB, P.O. Box 17192, S-104 62 Stockholm (SE).

(81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: REGENERATION OF BIOSENSORS



(57) Abstract

The invention comprises a method of regenerating a biosensor. It involves passing a background flow of fluid without reactive components through the flow passage. At a selected point in time a sample aliquot is injected into said background flow. At a point in time when a signal from said sensor is obtained the flow rate of the background fluid is increased. The invention also comprises a system for continuous monitoring of analytes in a biological fluid, the system having increased life by virtue of inherent regeneration of sensors employed. It comprises a biosensor (26, 30, 32), a sampling device (4) for providing a sample of said biological fluid, and means (10, 15, 18, 24) for passing a flow of a background fluid through said flow passage at selectable flow rates, means (20, 50, 55) for injecting said sample into said flow of background fluid, and means (50, 55) for increasing the flow rate of said combined flow. Means for achieving a washing action at the signal generating portion are provided.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01352

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/00, C12M 1/40
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	31 -10- 1998
Name and mailing address of the ISA/ Sw dish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12Q 1/00, C12M 1/40

A1

(11) International Publication Number: **WO 99/02723**

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/SE98/01352

(22) International Filing Date: 8 July 1998 (08.07.98)

(30) Priority Data:
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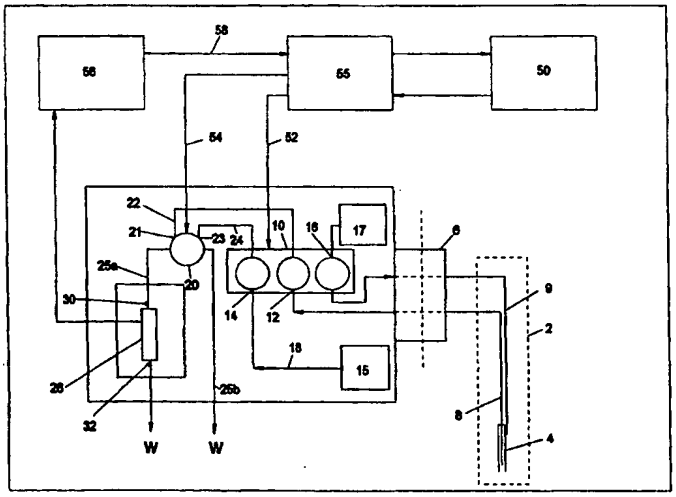
(71)(72) Applicant and Inventor: CARLSSON, Thomas [SE/SE]; Flöjtvägen 59, S-756 54 Uppsala (SE).

(74) Agents: LINDGREN, Anders et al.; Dr. Ludwig Brann Patentbyrå AB, P.O. Box 17192, S-104 62 Stockholm (SE).

(81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: REGENERATION OF BIOSENSORS



(57) Abstract

The invention comprises a method of regenerating a biosensor. It involves passing a background flow of fluid without reactive components through the flow passage. At a selected point in time a sample aliquot is injected into said background flow. At a point in time when a signal from said sensor is obtained the flow rate of the background fluid is increased. The invention also comprises a system for continuous monitoring of analytes in a biological fluid, the system having increased life by virtue of inherent regeneration of sensors employed. It comprises a biosensor (26, 30, 32), a sampling device (4) for providing a sample of said biological fluid, and means (10, 15, 18, 24) for passing a flow of a background fluid through said flow passage at selectable flow rates, means (20, 50, 55) for injecting said sample into said flow of background fluid, and means (50, 55) for increasing the flow rate of said combined flow. Means for achieving a washing action at the signal generating portion are provided.

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REGENERATION OF BIOSENSORS

The present invention relates in general to the field of biosensors, and in particular to methods and apparatus for regenerating such sensors, thereby increasing the effective life thereof.

In a specific aspect the invention relates to a system for continuous analysis of analytes in blood or serum comprising means for regeneration of the sensor employed therein.

Background of the Invention

Measurements of analytes in blood is commonly performed by sampling blood from patients and analyzing said samples in a laboratory, often situated at a location remote from the ward. E.g. for glucose analysis there are available special reagent sticks usable for measuring on site i.e. in the ward. However, the accuracy of such measurements is questionable, and the error could be 10 - 20% at best.

Often it is necessary to perform several sequential measurements over periods of several hours, which is very labor intensive. Furthermore, the risk for errors because of the human intervention is evident, and the low accuracy is of course also a drawback in this regard.

For the purposes of this application, the term "biosensor" means any device having a portion which interacts with biological or biochemical material, and has the capability to generate a signal indicative of a change in some parameter of said biological or biochemical material as a consequence of said interaction.

When analytes such as glucose, urea, lactate, ATP, glycerol, creatinine and pyruvate in biological samples, such as blood, plasma or serum are analyzed using biosensor techniques based on immobilization of enzymes, the sensor surface will be exposed to a certain amount of sample during a certain time sufficient to achieve an adequate sensor response. It is well known that the sensor response gradually will degrade because of fouling of the surface. This in its turn is a consequence of said exposure and the interaction between the surface and the substances present in the sample that occurs. The chemical and physical composition of the sample is thereby of importance, the sample i.a. comprising red cells, blood platelets, macromolecules, electrolytes, lipids, red/ox-compounds etc. It is also known that the support material for the enzyme immobilization in biosensors based on enzyme column technology is fouled by the substances present in the sample.

In cases where selective membranes are used for protection of the sensor surface of biosensors based on enzyme electrode technology, said membranes are also fouled by such substances. This fouling influences the sensor response by substantially reducing the life and stability of the biosensor.

Description of Related Art

Most known metabolite sensors today are based on the amperometric principle, that is measurement of oxygen consumption or hydrogen peroxide production in electrochemical reactions. However, interference with reducing/oxidizing substances causes problems like long time drift, need for frequent calibration and short life. Regarding the sampling procedure there exist devices which, before the actual

measurement, condition the blood before it enters the actual sensor by e.g. introducing a special step, such as dialysis. This is both a more complicated solution and also more expensive, since the dialysis cassette has to be replaced before a new measurement can be made.

Another known sensor principle is by utilizing the heat production when the analytes are decomposed by the appropriate enzyme for the analyte in question. This so called enzyme calorimeter principle is known from US-4,021,307. The enzyme calorimeter disclosed therein is however not suited for direct measurement on whole blood, since the blood cells quickly will clog the column containing the immobilized enzyme, due to adsorption of blood constituents such as various cells, trombocytes, proteins etc. This effect could be circumvented to a certain extent by diluting the blood at least ten times, which will reduce the sensitivity of the measurement considerably. However such a measure would require an extra supply of a diluting solution. Another way of reducing the clogging of the column is to use a special super porous support material with a pore size larger than 10 μm. This support made from agarose, is however softer than the conventional support materials used in this field, preferably glass, and therefore are at a certain risk of (occasionally) being compressed by the blood sample, which in turn quickly will clog the column.

Thus, at present there is no reliable method and system available for the direct and continuous analysis of whole blood drawn from patients.

Summary of the Invention

The present invention therefore seeks to provide an improved method of analyzing whole blood in respect of analytes such as glucose, lactate, urea, ATP, glycerol, creatinine and pyruvate wherein the drawbacks of the prior art methods are alleviated.

In particular the active life of a biosensor that is used for such analysis is prolonged by providing for reduced fouling of the sensor by regenerating the sensor in accordance with the invention.

The method according to the invention is defined in claim 1.

In a second aspect of the invention there is also provided a system for long time measurements of whole blood directly and continuously sampled from a patient, wherein the flow of the sampled blood is held at a very low rate.

The system according to the invention is defined in claim 12.

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying

drawings which are given by way of illustration only, and thus not limitative of the present invention, and wherein

Fig. 1 is an overview of a system according to the invention;

Fig. 2 is a cross sectional view through a connector according to the invention;

Fig. 3 is a view similar to Fig. 2, but showing a conventional connector without the inventive sealing feature; and

Fig. 4 is a flow chart illustrating the sequence of steps in the method of the invention.

Detailed Description of Preferred Embodiments

In Fig. 1 there is disclosed a system for performing a continuous monitoring of the concentration of analytes in blood.

It comprises a blood sampling device 2, such as a cannula 4 inserted in a vein of a patient. The cannula 4 is connected to the other system components via a tubing 8 and a suitable connector 6 (to be described) connecting to a pump 10, which is provided for drawing the various fluids through the system at controlled flow rates. The pump is a multichannel pump, and has a first input 12 for the blood from the sampling device 2, a second input 14 for buffer solution from a buffer storage 15, and a third input 16 for anticoagulant from an anticoagulant

reservoir 17. Anticoagulant is fed through a line 9 into the sample flow in line 8 at a point near the tip of the catheter 4.

Alternatively there may be provided separate pumps for the various components.

The valve 20 is important for the operation in accordance with the invention, and has two inputs, one input 21 for sample blood, fed from pump 10 through line 22, and one input 23 for buffer, fed through line 24. There are also two outputs, a first connecting to line 25a feeding the fluid to the analysis portion, and a second connecting to line 25b for discharging the fluid as waste. The valve 20 is designed such as to permit fluid (i.e. blood in this embodiment) from line 22 to be injected into the buffer flow from line 24.

All surfaces in the system exposed to sample are coated with heparin in order to make the system blood compatible.

The actual sample analysis may be carried out in a so called enzyme reactor (ER) 26, although it is contemplated that any biosensor type may be used, provided it has a sensitive portion arranged in some kind of flow passage where flow past the sensitive portion of the sensor can be controlled. Thus, a sensor of a type that is merely immersed in a liquid would not be suitable for use with this invention. An ER is used in a preferred embodiment and will be described in more detail below.

The system also comprises a control unit 50, which may be a micro processor or a PC. An interface 55 is connected between the control unit 50 and the components in the system, such that control signals to the pump 10 and the valve 20 are fed via

lines 52 and 54 respectively. Thus, the pumping rate in the various independently operated flow passages may be increased or decreased, and the valve 20 may be switched between its various positions by commands issued by the control unit in response to signals from the biosensor. An amplifier 56 is provided for amplifying the signals from the biosensor 26, and feeding said signals to the interface on line 58, for further transmission to the control unit which uses the information thus obtained to issue the appropriate control commands to the pump and valve.

The Enzyme Reactor

An enzyme reactor (ER) 26 comprises a sensor column. The column contains support material such as beads of glass or hard polymer resin, on which enzyme has been immobilized. Immobilization of enzyme is standard procedure and does not form part of this invention, and will hence not be described in detail herein.

The operation and function of the ER 26 is as follows.

The sensor column has two thermistors 30, 32, one 30 arranged at the column inlet and the other 32 at the column outlet. Fluid entering the column will begin reacting with the enzyme that is immobilized on the beads in the column, and will thereby generate heat, causing the temperature of the fluid to increase. By monitoring the temperatures at the inlet and outlet respectively, and integrating the temperature over time, the integral obtained will correspond to the heat of reaction, which then may be related to the concentration of e.g. glucose in the fluid.

Because of non-specific reactions that may be exothermic or endothermic and which occur in the column, temperature fluctuation must be accounted for. Thermostating the reactor is one way of doing this, but it can be achieved also in other ways and by other means, and is not crucial to the invention.

Operation

Returning now to Fig. 1, there is illustrated an embodiment of the system which comprises a biosensor 26 arranged in a thermostated environment. The system is operated as follows:

The catheter 4 is inserted in a blood vessel of a patient, and connected to the tubing of the system by means of a connector 6 (to be described). Initially the pump will draw blood through line 8 via line 22, through the valve 20 and to the waste line 25b for disposal, and buffer from buffer storage 15 is drawn via line 18 through the valve 20 and via line 25a into the enzyme reactor 26. The continuously measured signals obtained from the sensor when the buffer passes through it will form a background or zero level, and the flow of buffer will be referred to as a "background flow" in this application. The rate of background flow may vary in the range 0.1-10 ml/min., and preferably is 1 ml/min.

If desired, and indeed it is mostly required, anticoagulant is mixed with the blood. Normally a ratio between sample and anticoagulant of 1:1 will be used, although other ratios are conceivable for specific conditions. The anticoagulant is pumped in line 9 and injected in the sample flow line 8 near the catheter 4 tip.

At a time when it is desired to make a measurement the valve is given a "SWITCH TO INJECTION MODE" command to the effect that the blood is redirected into the buffer stream, for a period of time of a duration sufficient for an aliquot of 10 µl to be entered as a liquid plug in the buffer stream (other sample volumes may of course be employed, but at the present time 10 µl has proven suitable in most cases). This "blood plug" is passed in line 25a, which runs in the thermostated medium, where the sample obtains a controlled temperature, and then it enters the ER 26.

As soon as the blood, containing e.g. glucose, reaches the ER 26, the glucose will start reacting with the enzyme, thereby evolving heat of reaction. The enzyme reaction is a very rapid process. Other components in the blood, such as various cells, trombocytes and proteins having a tendency to adsorb to the material inside the reactor, will begin to adsorb. The latter process is however a slow process compared to the diffusion controlled enzyme reaction. The small glucose molecules diffuse very much faster than the macromolecules and other macro components in the blood.

The thermistor 32 at the output end of the ER 26 will experience a rise in temperature caused by the enzyme reaction occurring in the reactor (at this time the entire sample preferably should have entered the reactor, although this is not absolutely necessary, as will be discussed below).

In the present embodiment the temperature increase sensed by thermistor 32 is transmitted to the control unit which is programmed to respond to an increase in the temperature signal to issue a INCREASE BUFFER FLOW RATE command to the pump 10 to

increase the rate of flow of the buffer by 5-100%, preferably by 10-50%, most preferably by 15-30%.

By balancing the flow rates, i.e. defining a suitable ratio between background flow rate and increased flow rate, it is possible to create a situation where larger components, such as cells, proteins etc, are washed away before they have had an opportunity to adsorb on the active surfaces inside the ER 26, and at the same time allow the smaller molecules of interest sufficient time to react with the enzyme to such an extent that it is possible to detect the reaction.

This balancing of flow ratios within the given limits is made by straight forward routine experimentation for a given system, and is easily done by the skilled man.

In an alternative embodiment it may be sufficient if only a fraction of the sample has entered the reactor. In this case the detection of signal onset is not used for triggering. Instead a certain time is determined empirically, namely the time it takes for the sample to just about reach the reactor after injection into the background flow. This time is then programmed into the control unit and used as a starting point for increaseded flow. This time can of course be selected such that different fractions of sample enter the reactor. It should be noted that if only a very minor fraction has entered when increaseded flow is initiated, the signal will be low; however, in most cases the entire sample will have reached the reactor by virtue of the void volume of the reactor being substantially larger than the sample volume.

It could also be possible to wait a short time, such as up to 5 seconds, after the sample completely has entered the reactor, i.e. after the detection by thermistor 32, before increasing the flow. Thus, in fact there is a time interval during which increased flow can be performed. The actual set of parameters has to be found empirically for each individual system, and the skilled man will be able to find these parameters without inventive work.

The flow pulse at the higher flow rate is maintained until a preselected signal value from the reaction response has been recorded, e.g. a peak maximum, and at this point the flow will be decreased by a RETURN TO NORMAL FLOW command to the pump 10, thereby stopping the additional flow of buffer solution. The duration of the pulse of increased flow rate may be 10-60 s, preferably 20-40 s.

Temperature fluctuations may be eliminated by thermostating the system, e.g. having the ER 26 immersed in a controlled temperature bath.

Fig. 4 illustrates the control algorithm in a simplified flow chart form.

When it is desired to make a measurement, the operator may select a INJECT command from a menu, or the computer may be programmed to issue the command at a preselected point in time. This command will set the valve such that the flow of sample (blood) is diverted into the buffer flow, for a time sufficient to inject the desired sample volume, i.e. 10 µl. Then, the valve is reset to normal mode, i.e. the blood is discharged as waste.

If the system parameters, such as configurations, flows etc. are well defined, then it is possible to preset the time T when increased flow is to be initiated. Thus, when the elapsed time t after injection of sample equals T, increasing is initiated by the computer.

Alternatively, the computer continuously registers the signal from the thermistor, and when a signal gradient, i.e. a temperature rise, of sufficient magnitude occurs, increasing the flow is initiated.

The increase in flow is performed by increasing the pump speed, by the computer issuing a INCREASE PUMP SPEED command to the interface, such the initial pump rate P_0 is increased by a factor corresponding to an increase of 5-100%, preferably by 10-50%, most preferably by 15-30%. Thus, the pump speed during increased flow (or pulse) mode is

$$P = P_0 + XP_0.$$

After a time Δt when the reaction in the reactor is complete, the pump speed is reverted back to the initial value P_0.

Then, control reverts to the computer for either a programmed new measurement at a preselcted point in time, or an operator initiated measurement.

The Connector

In fig. 2 a connector device for connecting to a patient, suitable for use in connection with the system according to the invention is illustrated.

The connector device, generally designated 100, comprises a male part, generally designated 102, and a female part, generally designated 104.

The male part 102 is provided on the distal end of a catheter 106 that has been inserted in e.g. a blood vessel of a patient.

The female part 104 comprises a narrow tube 108 of e.g. steel, the inner diameter of which is larger than the inner diameter of the lumen 110 of the catheter 106. The ratio between diameters is preferably 2-3:1. Furthermore, the steel tube 108 is milled or ground on its outer proximal end such that a sharp cutting edge 112 is formed, i.e. the outer surface is made slightly conical at the proximal end.

The tube 108 is inserted in and fixed centrally of a concentric socket structure 114, forming said female part 104. The socket 114 thus comprises a cylinderlike element having a circular/cylindrical opening or bore 116, the inner surface of which is slightly tapered, and in the center of which the tube 108 protrudes a fractional distance of the depth of said opening. Thus the cutting edge 112 of the tube 108 is located somewhere in the region between the bottom 118 of said opening 116 and its peripheral edge 120. The tapering is such that the diameter at the bottom 118 is slightly smaller than the diameter at the peripheral edge 120.

Similarly, the catheter is located centrally of a cylindrical member 122 forming the male part 102, the outer diameter of which snugly fits inside the opening of the female part 104. The end surface 124 of the catheter 106 is flush with the end

surface 126 of the cylindrical member 122. The tube 108 extends so much away from the bottom 118 of the female part that when the male and female parts are connected, the sharp edge 112 will penetrate the end surface of the catheter 106.

The catheter 106 is made of a material that is enough resilient or soft, that when the male and female parts are connected, the cutting edge 112 sinks into the end surface 124 of the catheter 106. Thereby a reliable and safe connection is provided in the transport of blood from the patient to the measuring system. Suitable materials for the catheter are e.g. soft PVC/silicone.

An example of a suitable locking device usable with the connector and having a male/female structure as outlined above is a Luer®-type lock.

As can be seen in the figure there is an abrupt change in the flow cross-section at the connection between catheter 106 and tube 108. This is essential in the sense that it will prevent or alleviate clogging of the flow path. This principle is known.

By providing the direct contact connection between tube 108 and catheter 106, as disclosed above, the large volume 116 is eliminated from the flow path. This is important in the sense that if the blood would have to pass such a large volume before entering the tube 108, there would be enough time for the constituents of the blood to adhere to the inner surfaces of said volume 116. In Fig. 3 a connector comprising an ordinary Luer-lock type coupling is shown. The male part 102 and the female part 104 are connected such as to form a dead space 119. It is self evident that the flow rate will decrease drastically

when the blood enters the large dead space 119 inside the coupling, thereby giving the blood constituents time to adhere to the inner surface of the connector and eventually clog the connector.

Of course it is equally conceivable to provide the catheter in the female part and the tube in the male part. However, the first embodiment is preferred since the sharp edge of the tube will be protected if arranged "inside" the female part, as shown.

Other types of couplings are of course conceivable, the important feature is the provision of a sharp edge on the tube, and a catheter having the necessary softness or resiliency that the edge will actually sink into the material when the parts are connected.

For example one could envisage some type of screw and nut connector, or a bayonet type coupling.

The invention will now be further illustrated by way of the following non-limiting Examples.

EXAMPLES

The following Examples were performed with the setup shown in Fig. 1. The sensor was an enzyme reactor having dimensions 20 mm length x 4 mm diameter.

The skilled man will easily be able to select suitable thermistors having the appropriate properties. One example of thermistor is obtained from Victory Eng. Inc.

The sample volume was 5 or 10 µl, and the background flow was 1 ml/min.

Signal values are given in Volts.

Example I (comparative)

In this example the flow was kept constant, and thus no increased flow was applied. The sample (blood) volume was 5 µl, and the base line signal was recorded before and after detection was made. Three consecutive runs were performed.

	Signal/V	
Sample No.	Before det.	After det.
1	0,10	0,15
2	0,15	0,23
3	0,22	0,34

As is clearly demonstrated the baseline signal before detection increases from 0,10 to 0,22 V, and also the baseline signal after detection increases from 0,15 to 0,34 V.

Example II (comparative)

The experiment of Example I was repeated with a fresh sensor and new samples.

Sample No.	Before det.	After det.
4	0,14	0,38
5	0,35	0,63

Again the base line signals clearly are not reproducible between runs.

Example III (comparative)

In this example the flow was also kept constant but a sample prepared from a standard solution and glucose was introduced, and passed through the reactor.

Sample No.	Before det.	After det.
6	0,30	0,31
7	0,29	0,31

As can be seen, the base line signal is not affected.

Example IV (comparative)

The same conditions as in Example I, but the sample volume is increased to 10 µl.

Sample No.	Before det.	After det.
8	0,55	0,64
9	0,59	0,67
10	0,62	0,70

Again, the base line is not reproducible between runs.

Example V (according to the invention)

In this example the sample volume was 10 µl. When the onset of sensor response was detected, the buffer flow was increased by

15% and maintained at that level for 20 seconds, when the response signal began to decrease again.

Sample No.	Before det.	After det.
11	0,23	0,22
12	0,23	0,22
13	0,23	0,22
14	0,22	0,22
15	0,21	0,22
16	0,20	0,22
17	0,21	0,22
18	0,21	0,21
19	0,23	0,21

As can be seen from the table, 9 consecutive runs were made and the base line returned reproducibly to the same level within the accuracy that measurements allow.

The system described herein is preferably designed as a " bed-side monitor" , i.e. a portable system for making around-the-clock surveillance of e.g. intensive care patients, or patients undergoing dialysis.

The control unit and other hardware components are thereby integrated in one single piece of equipment that is easily moved from one location to another.

Although the description has been made with reference to a system and method for analyzing analytes in blood, it is equally possible to use the inventive ideas for other types of complex biological/biochemical media, such as fermentation media, animal cell culture media.

For example it is suitable for analyzing ethanol or residual sugar in mash in brewing processes. It could also be used for analyzing various substances, e.g. insulin, amino acids or growth hormone in cell culture media.

It could also be used to analyze various components in milk or similar foodstuffs.

The skilled man could envisage numerous other applications of the basic principle of the invention, and implement them without inventive work.

Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

CLAIMS:

1. A method of regenerating a biosensor of the type having a signal generating portion responsive to some property of or to the presence of some component in a biological fluid, and having a flow passage through which fluid is being passed at selectable flow rates, the method comprising:

a) passing a background flow of fluid without response generating components through the flow passage;

b) at a selected point in time introducing a sample aliquot into said background flow;

characterized by

c) increasing the flow rate of the background fluid at a point in time when at least a fraction of said sample aliquot has entered said flow passage.

2. The method of claim 1, comprising detecting the presence of the sample by the sensor and increasing the flow rate at a point in time 0-30 seconds after the presence of sample is detected, preferably 0-20 seconds, more preferably 0-10 seconds, and most preferably immediately after such detection.

3. The method of claim 1 or 2, wherein said flow rate is increased by 5-100%, preferably 10-50%, most preferably 15-30%.

4. The method of claim 1 or 2, comprising maintaining the increased flow rate until the signal from the sensor has reached a preselected value.

5. The method of claim 4, wherein said preselected value is a signal peak maximum.

6. The method of any preceding claim, wherein the increased flow rate is maintained for 10-60 s, preferably 20-40 s.

7. The method of any preceding claim, wherein said background flow is 0.1 - 10 ml/min., preferably 1 ml/min.

8. The method of any preceding claim, wherein said increase in flow rate is initiated when the entire sample has entered said flow passage.

9. The method of any preceding claim, wherein sample is continuously drawn from a sample source, and when not being analyzed it is disposed as waste.

10. The method of any preceding claim, wherein the sample is blood, optionally premixed with anticoagulant.

11. The method of claim 10 wherein said anticoagulant is premixed with blood in a ratio of 1:1.

12. A system for continuous monitoring of analytes in a biological fluid, the system having increased life by virtue of inherent regeneration of sensors employed, the system comprising

a) a biosensor (26, 30, 32) of the type having a flow passage through which fluid is being passed at selectable flow rates, and a signal generating portion located in said flow passage and responsive to some component or property of a biological fluid,

b) a sampling device (4) for providing a sample of said biological fluid;

c) means (10, 15, 18,24) for passing a flow of a background fluid through said flow passage at selectable flow rates;

d) means (20, 50, 55) for injecting said sample into said flow of background fluid at selectable points in time to provide a combined flow;

e) means (50, 55) for increasing the flow rate of said combined flow at a selectable point in time during passage of the sample through said flow passage in order to achieve a washing action on the signal generating portion; and

f) means (30, 32) for providing a signal from said signal generating portion.

13. The system of claim 12, wherein said sampling device comprises a catheter (4) insertable in a blood vessel of a human or an animal, and tubing (8) connecting the catheter to the system.

14. The system of claim 12 or 13, wherein said means for passing a flow of a background fluid through said flow passage at selectable flow rates comprises a pump (10) and appropriate tubing (18, 24).

15. The system of claim 12, 13 or 14, wherein said means for injecting said sample into said flow of background fluid, comprises a valve (20) switchable between injection and waste disposal modes.

16. The system of any of claims 12-15, wherein said means for increasing the flow rate comprises a control unit (50) programmed to respond to signals from said sensor.

17. The system of any of claims 12-16, wherein said means for providing a signal from said signal generating portion comprises at least one thermistor (30, 32).

18. The system of any of claims 12-17, further comprising a connector (100) for connecting said sampling device (4) to said pump (10), the connector comprising

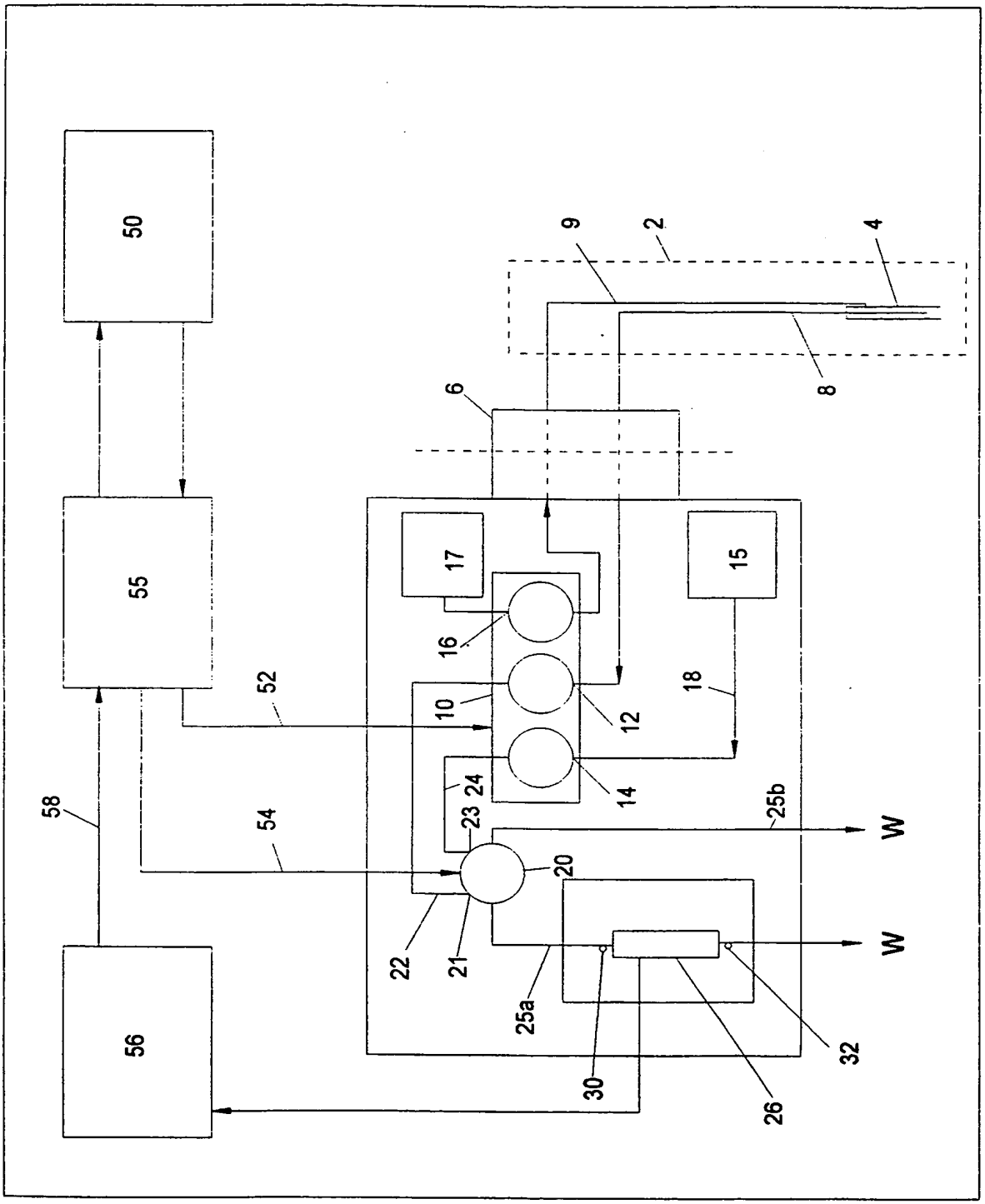
a male (102) and a female (104) part,

a tube (108) of a hard material such as steel having an inner diameter, and being inserted in the center of one of said male (102) and female (104) parts and protruding from an end surface (118) of said part (104; 102),

a catheter (106) of a soft material inserted in the center of the other of said male (102) and female (104) parts and having an inner diameter substantially smaller than the inner diameter of said tube (108), and having an essentially flat end surface (126), wherein

the protruding end of said tube is ground such as to form a sharp circumferential edge (112), and wherein

the positions of said tube (108) and said catheter (106) in their respective male or female part, are such that when said male and female parts are connected, said sharp edge (112) penetrates into said catheter (106), thereby forming a fluid tight connection.


50
55
56
58
52
54
9
2
4
8
6
17
15
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18
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24
23
25b
W
W
20
22
21
25a
30
26
32

Fig 1

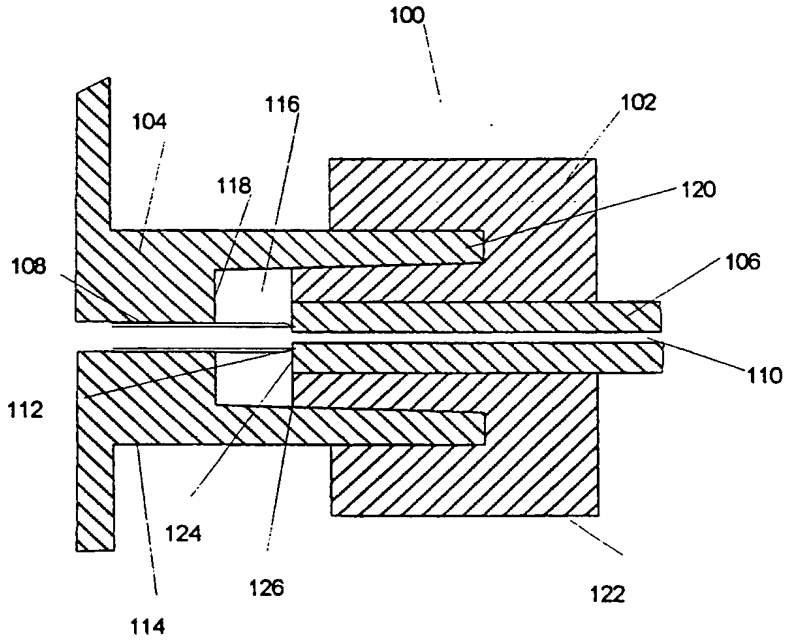

Fig 2

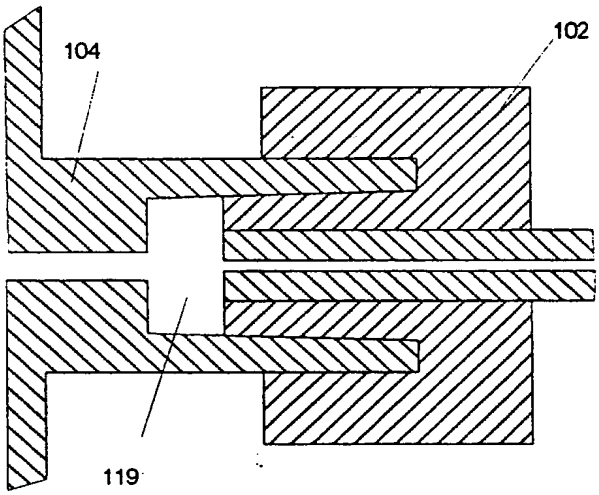

Fig 3

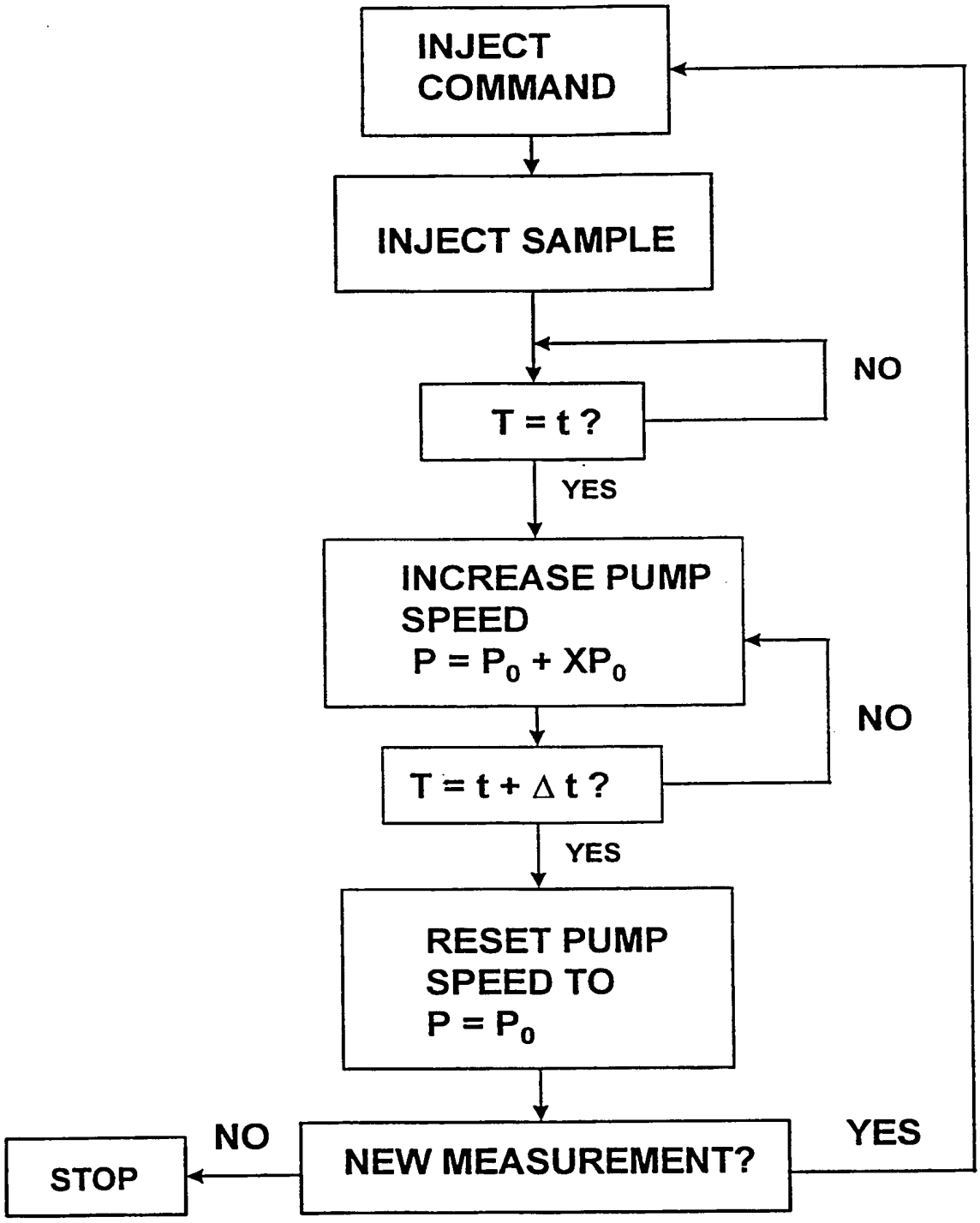
INJECT COMMAND
INJECT SAMPLE
T = t ?
NO
YES
INCREASE PUMP SPEED
P = P0 + XP0
T = t + Δ t ?
NO
YES
RESET PUMP SPEED TO
P = P0
NEW MEASUREMENT?
NO
YES
STOP

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01352

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/00, C12M 1/40
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12Q, C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4153513 A (HERMANN EDELMANN ET AL), 8 May 1979 (08.05.79) -- --------	1-18

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	31 -10- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/SE 98/01352

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